IMMULITE®

Anti-HBc IgM

IgM Antibodies to Hepatitis B Core Antigen

DPC

IMMULITE® Anti-HBc IgM

WARNING

This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

Performance characteristics have not been adequately established for use of the IMMULITE Anti-HBc IgM assay in infants, children, or adolescents.

Federal law restricts this device to sale by or on the order of a physician.

Assay performance characteristics have not been established when the IMMULITE Anti-HBc IgM assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

Intended Use: IMMULITE Anti-HBc IgM is a solid-phase chemiluminescent enzyme immunoassay designed for use on the IMMULITE automated immunoassay analyzer for the qualitative measurement of IgM antibody to hepatitis B core antigen (anti-HBc IgM) in human serum or plasma (EDTA, heparinized, or citrate). It is intended for *in vitro* diagnostic use for the presumptive laboratory diagnosis of acute or recent (usually within six months) hepatitis B viral infection.

Caution: Performance characteristics for the IMMULITE Anti-HBc IgM were determined in part using archival specimens. Laboratories are advised that they should monitor results using other DPC HBV serological markers or retest questionable specimens with IMMULITE Anti-HBc IgM or another legally-marketed Anti-HBc IgM assay.

Catalog Number: **LKMC1** (100 tests), **LKMC5** (500 tests)

Test Code: **BcM** Color: **Aqua**

Summary and Explanation

Hepatitis B virus (HBV) is the sole human pathogen in the family of Hepadnaviridiae, a DNA virus, and is found world-wide. Distribution of HBV infection will vary among geographical areas and population groups. Transmission of the virus is due to parenteral contact, through the exchange of blood or blood products, sexual contact, and perinatal spread from mother to newborn.[1,2] Clinical manifestations range from mild asymptomatic infections to severe fulminant hepatitis.[1,2,3] Over 90% of infected adults will have an acute self-limiting infection,[2] with approximately 30% of these individuals developing jaundice, liver enzyme elevation, and symptoms. Recovery occurs without any chronic sequelae.[1,2]

Chronic liver disease, a condition in which infection persists for more than six months, a known sequela of a hepatitis B infection, is usually progressive.[1,2] The risk of developing the chronic carrier state is more likely to follow infection acquired in childhood than as an adult.[4,5] In chronic HBV carriers, there is no evidence of continued hepatic damage,[1,2] however, the infection persists and the carrier maintains the ability to transmit the virus.[2]

Availability of HBV vaccines, and the recommendation of universal immunization for infants and other high-risk persons has aided in the prevention of HBV infections. In addition, treatment with alpha-interferon to relieve symptoms is available. Results have shown positive response to treatment in 40–50% of selected individuals with chronic active hepatitis B.[4,5]

Classification of a hepatitis B infection requires the identification of several serological markers expressed during three phases (incubation, acute and convalescent) of the infection. The first marker to appear during the incubation phase is HBsAg, and indicates an ongoing infection with HBV.[1,2,4] IgM and total anti-HBc appears shortly after the appearance of HBsAg, when the individual usually becomes symptomatic, and peaks during the acute phase prior to the appearance of anti-HBs. IgM antibody to the core antigen will decline in uncomplicated acute infection, whereas IgG antibody will persist for years.[4,5] IgM and total anti-HBc may also be elevated in chronic HBV infections.[4]

22

Presence of IgM and total anti-HBc indicates an ongoing or recent HBV infection. When used in conjunction with tests for other HBV serological markers, a laboratory diagnosis or a rule out of HBV infection can be achieved.

Principle of the Procedure

IMMULITE Anti-HBc IgM is a solid-phase, two-step chemiluminescent enzyme IgM antibody capture immunoassay. The solid phase, a polystyrene bead enclosed within a Test Unit, is coated with a monoclonal murine anti-IgM antibody.

The patient specimen is added to the Test Unit containing a coated bead. An alkaline phosphatase-labeled recombinant HBc antigen is also added to the Test Unit. After the wash and incubation steps, chemiluminescent substrate undergoes hydrolysis in the presence of alkaline phosphatase. IMMULITE Anti-HBc IgM is an immunometric assay. The photon output, as measured by the luminometer, is related to the presence of HBc IgM antibodies in the sample.

Incubation Cycles: 2 × 30 minutes.

Specimen Collection

Hemolyzed samples may indicate mistreatment of a specimen before receipt by the laboratory; hence, the results should be interpreted with caution.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Samples should be thoroughly separated from all cellular material. Failure to do so may lead to a falsely elevated results.[9]

The IMMULITE Anti-HBc IgM assay may be performed on human serum or plasma (heparinized, sodium citrate or EDTA).

If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.

Specimens containing precipitate may give inconsistent test results. To prevent this problem, such specimens should be clarified prior to assaying. Specimens showing particulate matter or red blood cells may give inconsistent results and should be centrifuged prior to testing (recommended 8,000 to 10,000 RCF × 10 minutes). Specimens, which are not tested within 24 hours, should be removed from the clot or red blood cells.

Do not use heat-inactivated specimens.

Specimens with obvious microbial contamination should not be tested.

More than two freeze-thaw cycles are not recommended.

Volume Required: 10 µL serum or plasma (heparinized, sodium citrate or EDTA). (Sample cup must contain at least 100 µL more than the total volume required.)

Storage: 2 days at room temperature (15°–28°C).[10]
3 days at 2–8°C.[8,10]
For longer storage: at –20°C.[7]

Patient Sample Dilution: Samples must be prediluted 1-in-21 in Anti-HBc IgM Sample Diluent (LMCZ1, LMCZ2), e.g. by adding 20 µL sample to 400 µL Diluent. Mix well to ensure a homogeneous solution.

Note that printed results have been corrected for the 1-in-21 dilution.

Warnings and Precautions

For *in vitro* diagnostic use.

This assay was designed and validated for use with human serum or EDTA, heparin, and Na Citrate plasma from individual patient specimens. Pooled specimens must not be used.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Because no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents, specimens should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual,

23

Biosafety in Microbiological and Biomedical Laboratories, 1993.

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested by FDA recommended test procedures and found nonreactive for syphilis; for antibodies to HIV1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

The Anti-HBc IgM Adjustor, Anti-HBc IgM Low Positive and Anti-HBc IgM Positive Controls contain HBcAg which may be indicative of low levels of HBV. These components have been inactivated by proven, documented methods. However, always handle all controls as if capable of transmitting infectious agents.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water for the wash step and to avoid system contamination.

Materials Supplied

Components are a matched set. The barcode labels are needed for the assay. Barcodes encode information about the test, including expiration dates, component lot numbers, adjustment parameters, and cutoff parameters.

Anti-HBc IgM Test Units (LMC1)
Each barcode-labeled unit contains one bead coated with a monoclonal murine anti-IgM antibody manufactured at DPC. Stable at 2–8°C until expiration date.
LKMC1: 100 units. **LKMC5:** 500 units.

Allow the Test Unit bags to come to room temperature before opening. Open by cutting along the top edge, leaving the ziplock ridge intact. Reseal the bags to protect from moisture.

Anti-HBc IgM Reagent Wedges (LMCA, LMCB)
With barcodes. **LMCA:** 6.5 mL of a protein-based buffer, with preservative. **LMCB:** 6.5 mL alkaline phosphatase (bovine calf intestine) conjugated to a purified recombinant HBcAg (produced in *E. coli* bacteria) in buffer, with < 0.1 g/dL sodium azide. Store capped and refrigerated: stable at 2–8°C until expiration date. Recommended usage is within 30 days after opening when stored as indicated.
LKMC1: 1 set. **LKMC5:** 5 sets.

Anti-HBc IgM Adjustors (LMCL, LMCH)
Two vials (Low and High) of lyophilized human serum reactive to HBcAg in buffer, with < 0.1 g/dL sodium azide. Reconstitute each vial with **2.0 mL** distilled or deionized water. Mix by gentle swirling or inversion until the lyophilized material is fully dissolved. (No further dilution is required.) Stable at 2–8°C for 14 days after reconstitution, or for 6 months (aliquoted) at –20°C.
LKMC1: 1 set. **LKMC5:** 2 sets.

Anti-HBc IgM Controls (LMCC1, LMCC2, LMCC3) (may be purchased separately.)
Three vials (Negative, Low Positive and Positive). **LMCC1 (Negative Control):** human serum nonreactive to HBcAg, with < 0.1 g/dL sodium azide. **LMCC2, LMCC3 (Low Positive Control, Positive Control):** human serum reactive to HBcAg, with < 0.1 g/dL sodium azide. Reconstitute each vial with **2.0 mL** distilled or deionized water. Mix by gentle swirling or inversion until the lyophilized material is fully dissolved. (No further dilution is required.) Stable at 2–8°C for 14 days after reconstitution, or for 6 months (aliquoted) at –20°C.
LKMC1: 1 set. **LKMC5:** 2 sets.

For the current control ranges, please refer to the Control insert.

Anti-HBc IgM Sample Diluent (LMCZ1*, LMCZ2†)
For the manual dilution of patient samples. Vials containing a buffer solution, with <0.1 g/dL sodium azide. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquoted) at –20°C.
***LKMC1:** 2 × 25 mL.
†LKMC5: 3 × 100 mL.

24

Kit Components Supplied Separately

LSUBX: Chemiluminescent Substrate
LPWS2: Probe Wash Module
LKPM : Probe Cleaning Kit
LCHx-y: Sample Cup Holders (barcoded)
LSCP: Sample Cups (disposable)
LSCC: Sample Cup Caps (optional)

Also Required
Sample transfer pipets, distilled or deionized water.

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in Section 6 of the IMMULITE Operator's Manual.

See Section 4 of the IMMULITE Operator's Manual for: preparation, setup, adjustment, assay and quality control procedures.

Visually inspect each Test Unit for the presence of a bead before loading it onto the system.

Note that both Reagent Wedges A and B must be loaded on the carousel to run this assay.

Adjustment Interval: 4 weeks. The adjustors are used to correlate the cps (counts per minute) of the customer's instrument to that of the master curve instrument.

Quality Control Samples: The control(s) supplied with the kit should be used as quality control material to monitor the performance of the assay.

The quality control material is in a serum matrix. It may not adequately control the assay for plasma matrix specimens. The user should provide alternate control material for a plasma matrix.

If control results fall outside the stated range, patient results should not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.

It is recommended that the user refer to *Internal Quality Control Testing: Principles and Definitions* and other published guidelines for general quality control recommendations and intervals for testing.[9,11]

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

The low positive control is used to verify that the test kit components are capable of detecting a reactive specimen, provided the test procedure has been strictly adhered to.

It is recommended that the controls supplied with the kit be used as quality control material to monitor the performance of the assay.

For the current control ranges, please refer to the Control insert.

Calibration

The IMMULITE Anti-HBc IgM calibration employs a stored master curve, generated by the four-parameter logistic mathematical model based on the dose-cps (counts per minute) relationship during the calibration process.

DPC's IMMULITE Anti-HBc IgM is calibrated against the Anti-HBc IgM reference serum from Paul Ehrlich Institute (PEI). The cutoff of the assay was set at 10 U/mL based on an ROC analysis from 130 anti-HBc IgM positive (spiked and endogenous) and negative (endogenous) specimens and on subsequent cutoff validation studies.

Interpretation of Results

Positive: A result greater than, or equal to 11 U/mL is considered to be "presumptively positive" for IgM antibody to HBcAg, and is presumptive evidence of recent infection with HBV. Further testing is required with other HBV serological markers to confirm the HBV infection state.

Negative: A result less than 9 U/mL is considered to be "negative" and is indicative that the patient has not been recently infected with HBV. Negative results by this test do not preclude recent primary infection. To define the HBV infection state further testing is

25

recommended with other HBV serological markers.

Retest Zone: A result less than 11 U/mL and greater than or equal to 9 U/mL is indeterminate and must be retested in duplicate. If all three results (original and two retests) are greater than or equal to 10 U/mL, the specimen is positive for anti-HBc IgM. If all three results (original and two retests) are less than 10 U/mL, the specimen is negative for anti-HBc IgM.

A specimen that has been retested but cannot be resolved as above remains indeterminate. An indeterminate result (9-11 U/mL) should not be reported until it is retested and resolved. The immune status of the individual should be further assessed by associated risk factors and the use of additional diagnostic information, or another sample may be collected and tested.

It is recommended that patients exhibiting indeterminate IMMULITE Anti-HBc IgM results be closely monitored over time (approximately one week intervals) to determine if the IgM anti-HBc result becomes reactive, i.e., >11 (associated with acute hepatitis B infection), or non-reactive, i.e., <9 (associated with recovery).

For the determination of seroconversion, two sera or plasma (heparinized, sodium citrate or EDTA) samples should be drawn three to four weeks apart, during the acute and convalescent stages of the infection.

If U/mL values are reported, it is recommended that the following be included with the report:

> The following results were obtained with the IMMULITE IgM Anti-HBc EIA. Values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported total IgM anti-HBc level cannot be correlated to an endpoint titer. The clinical significance of values reported greater than or equal to 11 mIU/mL and less than 9 mIU/mL have not been determined, other than the individual is presumed to have been recently infected with HBV (≥ 11) or has not been recently infected with HBV (<9).

Limitations

The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall laboratory and clinical picture. False results may be obtained with any diagnostic test.

IMMULITE Anti-HBc IgM is limited to the detection of IgM anti-HBc in human serum or plasma. It can be used to furnish presumptive information as to whether a patient has, or has recently had, acute or subclinical hepatitis B infection. Supportive laboratory and clinical information, including other hepatitis B markers, must be used to determine the HBV disease state.

Assay performance characteristics have not been established for any specimen matrices other than serum, or heparinized, EDTA, or sodium citrate plasma.

Testing using alternative methodologies may be warranted if signs, symptoms, and risk factors are indicative of viral hepatitis, and other laboratory tests are nonreactive for the diagnosis of viral hepatitis.

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of this type of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Expected Values

Individuals acutely infected with the hepatitis B virus exhibit anti-HBc IgM between two weeks and four months after infection, usually through the course of clinical illness. IgM antibody levels decline in cases of uncomplicated acute infection, but remain elevated in chronic HBV infection.

Demographics and expected prevalence rates for apparently healthy, pre-vaccination, and pregnant subjects, each of whom provided one specimen, from

three clinical studies, one in the northwestern United States (Study 1), one in the southern United States (Study 4) and one in Europe, are summarized in the following table.

Subject	Total *n*	Male	Female	Mean Age	Age Range	Pos. by IML Anti-HBc IgM	
						n	%
Apparently Healthy (Europe)	200	Not available				0	0.0%
Pre-vaccination (Study 1)	17	8	9	34	21-56	0	0.0%
Pregnant at high risk[1] (Study 1)	3	0	3	33	30-36	0	0.0%
Pregnant at low risk[1] (Study 1)	16	0	16	31	21-42	0	0.0%
Pregnant at low risk[1] (Study 4)	195	0	195	28	17-41	1	0.5%
Pregnant at low risk[1] (Europe)	3	Not available				0	0.0%

[1] At high risk or low risk of exposure to hepatitis B.

Performance Characteristics

I. Clinical Performance

Four clinical studies with 769 specimens and one comparison study with 46 specimens from a total of 815 subjects were conducted to assess the performance of the IMMULITE Anti-HBc IgM assay.

The participating subjects each contributed one specimen to the analyses. The specimens were tested by FDA-approved or licensed hepatitis B assays (Reference markers) and IMMULITE Anti-HBc IgM. All specimens in the analyses were initial test results.

The data were analyzed following the assignment of specimen classification based upon the reactive(+)/ nonreactive(-) patterns for the HBV reference serological markers. Specimen classification was based only on the HBV serological marker results for that particular specimen. No other laboratory or clinical information was used in the disease classification process.

Study 1: Conducted in the northwestern United States, this study included 281 patients, consisting of 138 males and 88 females. Gender for 55 subjects was not reported. These subjects had an average age of 43 years, ranging from 21 to 85 years. Distributions of the ethnicity of the subjects are shown in the following table.

Ethnicity	*n*	%
African American	15	5.3%
Caucasian	169	60.1%
Hispanic	2	0.7%
Asian	32	11.4%
Other	3	1.1%
Unknown	60	21.4%
Total	281	100.0%

These subjects included acute and chronic hepatitis B patients, vaccinated individuals, pregnant women, and apparently healthy individuals, and patients with potentially crossreactive substances and medical conditions.

A total of 281 specimens were prospectively (n=92) and retrospectively (n=189) collected and tested by FDA-approved hepatitis B assays for six HBV serological markers (HBsAg, HBeAg, anti-HBc IgM, anti-HBc, anti-HBe, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated 17 unique patterns:

27

Characterization based on single point specimens	No. of patients	HBV Reference Markers					
		HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc	Anti-HBe	Anti-HBs
Acute	2	+	–	–	–	–	–
Acute	1	+	+	–	–	–	–
Acute	32	+	–	+/-	+	+	–
Acute	34	+	+	+/-	+	–	–
Chronic	2	+	–	–	+	–	–
Chronic	3	+	+/-	–	+	+	+
Chronic	1	+	–	–	+	–	+
Chronic	1	+	+	+/-	+	–	+
Early Recovery	16	–	–	+/-	+	+	+
Early Recovery	4	–	–	–	+	+	–
Early Recovery	19	–	–	–	+	+/-	–
Early Recovery	1	–	–	+	+	+/-	+
HBV vaccine response	27	–	–	–	–	–	+
Not previously infected	120	–	–	–	–	–	–
Recovered	16	–	–	–	+/-	–	+
Recovered	1	–	+/-	–	+	–	+
Uninterpretable	1	–	+	–	–	–	–

Based on the above classifications the IMMULITE Anti-HBc IgM results were compared to Kit A, a reference assay for the determination of anti-HBc IgM.

Reference	Kit A						Total
	+		Ind		–		
	IML Anti-HBc IgM						
	+	–	+	–	+	–	
HB Acute infection	6	0	3	0	6	54	69
HB Chronic infection	0	0	0	0	0	7	7
HB Early recovery	0	1	1	0	0	38	40
HBV Vaccine response	0	0	0	0	0	27	27
Not previously infected	0	0	0	0	0	120	120
Recovered	0	0	0	0	2	15	17
Uninterpretable	0	0	0	0	0	1	1
Total	6	1	4	0	8	262	281

HB Acute Infection
Positive agreement = 100.0% (6/6)
95% CI = 54.1% to 100.0%
Negative agreement = 90.0% (54/60)
95% CI = 79.5 to 96.2%

HB Chronic Infection
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (7/7)
95% CI = 59.0 to 100.0%

Early Recovery
Positive agreement = N/A (0/1)
95% CI = N/A
Negative agreement = 100.0% (38/38)
95% CI = 90.7 to 100.0%

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (27/27)
95% CI = 87.2 to 100.0%

Not previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (120/120)
95% CI = 97.0 to 100.0%

Recovered
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 88.2% (15/17)
95% CI = 63.6 to 98.5%

Uninterpretable
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (1/1)
95% CI = N/A

Total
Positive agreement = 85.6% (6/7)
95% CI = 42.1 to 99.6%
Negative agreement = 97.0% (262/270)
95% CI = 94.2 to 98.7%
Total agreement = 97.0% (268/277)
95% CI = 94.2 to 98.7%

Study 2: Conducted in the northeastern United States, this study included 209 patients, consisting of 104 males and 103 females. Gender for two patients was not reported. These patients had an average age of 47 years, ranging from newborn to 93 years. Distributions of the ethnicity of the patients are shown in the following table.

Ethnicity	*N*	%
African American	21	10.0%
Caucasian	101	48.3%
Hispanic	3	1.4%
Asian	6	2.9%
Other	7	3.3%
Unknown	71	34.0%
Total	209	100.0%

Included were patients with potentially cross-reactive substances and medical conditions.

A total of 209 retrospective specimens were collected and tested by FDA-approved or licensed hepatitis B assays for four HBV serological markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated eight unique patterns:

Characterization based on single point specimens	# of patients	HBV Reference Markers			
		HBsAg	Anti-HBc IgM	Anti-HBc	Anti-HBs
Acute	8	+	–	–	–
Acute	9	+	+/-	+	–
Chronic	2	+	–	+	+
Early recovery	33	–	+/-	+	+
Early recovery	17	–	–	+	–
HBV vaccine response	32	–	–	–	+
Not previously infected	107	–	–	–	–
Uninterpretable	1	+	–	–	+

Based on the above classifications the IMMULITE Anti-HBc IgM results were compared to Kit A.

Reference Serological Characterization	Kit A						Total
	+		Ind		–		
	IML Anti-HBc IgM						
	+	–	+	–	+	–	
HB Acute infection	2	0	1	0	1	13	17
HB Chronic infection	0	0	0	0	0	2	2
HB Early recovery	0	0	2	0	1	47	50
HBV Vaccine response	0	0	0	0	0	32	32
Not previously infected	0	0	0	0	0	107	107
Uninterpretable	0	0	0	0	0	1	1
Total	2	0	3	0	2	202	209

HB Acute Infection
Positive agreement = N/A (2/2)
95% CI = N/A
Negative agreement = 92.9% (13/14)
95% CI = 66.1 to 99.8%

HB Chronic Infection
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (2/2)
95% CI = N/A

Early Recovery
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 97.9% (47/48)
95% CI = 88.9 to 99.9%

29

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (32/32)
95% CI = 89.1 to 100.0%

Not previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (107/107)
95% CI = 96.6 to 100.0%

Uninterpretable
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (1/1)
95% CI = N/A

Total
Positive agreement = N/A (2/2)
95% CI = N/A
Negative agreement = 99.0% (202/204)
95% CI = 96.5 to 99.9%
Total agreement = 99.0% (204/206)
95% CI = 96.5 to 99.9%

Study 3: Specimens obtained from China were tested in the southern United States. This study included 79 patients and was comprised of 13 females and 55 males (gender for 11 patients was not reported) with an average age of 36 years, ranging from 18 to 82 years. These were prospectively recruited patients from a clinically well-characterized, homogeneous population: acute hepatitis B patients who presented with symptoms typical of acute hepatitis B such as jaundice, persistent fatigue, loss of appetite, pale stools and liver enlargement. Their ALT and AST results were significantly elevated at the time of diagnosis.

A total of 79 specimens were prospectively collected and tested by FDA-approved or licensed hepatitis B assays for six HBV serological markers (HBsAg, HBeAg, anti-HBc IgM, anti-HBc, anti-HBe, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated 11 unique patterns:

Characterization based on single point specimens	# pat	HBV Reference Markers					
		HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc	Anti-HBe	Anti-HBs
Acute	23	+	–	+/-	+	+	–
Acute	8	+	+/-	+	+	+	–
Acute	1	+	–	+	+/-	–	–
Acute	35	+	+	+/-	+	–	–
Acute	4	+	+	+	+	–	+/-
Chronic	1	+	–	–	+	–	–
Chronic	3	+	+/-	–	+	+	–
Chronic	1	+	+	+/-	+	–	+
Chronic	1	+	+	+	+	+	+
Recovered	1	–	–	–	+/-	–	+
Uninterpretable	1	+	–	+	+	+	+

Based on the above classifications the IMMULITE Anti-HBc IgM results were compared to Kit B, a reference assay for the determination of anti-HBc IgM.

Reference Serological Characterization	Kit B						Total
	+		Ind		–		
	IML Anti-HBc IgM						
	+	–	+	–	+	–	
HB Acute infection	29	4	3	0	12	23	71
HB Chronic infection	1	0	0	0	2	3	6
Recovered	0	0	0	0	0	1	1
Uninterpretable	1	0	0	0	0	0	1
Total	31	4	3	0	14	27	79

HB Acute Infection
Positive agreement = 87.9% (29/33)
95% CI = 71.8 to 96.6%
Negative agreement = 65.7% (23/35)
95% CI = 47.8 to 80.9%

HB Chronic Infection
Positive agreement = N/A (1/1)
95% CI = N/A
Negative agreement = 60.0% (3/5)
95% CI = 14.7 to 94.7%

Recovered
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (1/1)
95% CI = N/A

Uninterpretable
Positive agreement = N/A (1/1)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

Total
Positive agreement = 88.6% (31/35)
95% CI = 73.3 to 96.8%
Negative agreement = 65.9% (27/41)
95% CI = 49.4 to 79.9%
Total agreement = 76.3% (58/76)
95% CI = 65.2 to 85.3%

Study 4: Conducted in the southern United States, this study included retrospectively collected specimens from 200 pregnant subjects. These subjects had an average age of 28 years, ranging from 17 to 41 years.

A total of 200 specimens were tested by FDA-approved or licensed hepatitis B assays for four HBV serological markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated three unique patterns:

Characterization based on single point specimens	No. of subjects	HBV Reference Markers			
		HBsAg	Anti-HBc IgM	Anti-HBc	Anti-HBs
Early recovery	4	–	+/-	+	+
Early recovery	2	–	–	+	–
HBV vaccine response	42*	–	–	–	+
Not previously infected	152*	–	–	–	–

Based on the above classifications the IMMULITE Anti-HBc IgM results were compared to Kit B.

Reference Serological Characterization	Kit B						
	+		Ind		–		
	IML Anti-HBc IgM						
	+	–	+	–	+	–	Total
Early recovery	0	0	0	0	1	5	6
HBV vaccine response	0	0	0	0	0	41	41*
Not previously infected	0	0	0	0	0	148	148*
Total	0	0	0	0	1	194	195

* Five specimens were not tested for IMMULITE Anti-HBc IgM.

Early Recovery
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 83.3% (5/6)
95% CI = 35.9 to 99.6%

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (41/41)
95% CI = 91.4 to 100.0%

Not Previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (148/148)
95% CI = 97.5 to 100.0%

Total
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 99.5% (194/195)
95% CI = 97.2 to 100.0%
Total agreement = 99.5% (194/195)
95% CI = 97.2 to 100.0%

Study 5: In an additional study conducted in-house at DPC, IMMULITE Anti-HBc IgM was compared to DPC's IMMULITE 2000 Anti-HBc IgM on 46 samples. (Concentration range: approximately 2 to 95 U/mL. See "Method Comparison" graph.) By linear regression:

(IML 2000) = 0.99 (IML) – 0.34 U/mL
r = 0.981

Slope 95% CI: 0.93, 1.05

Intercept 95% CI: -3.22, 2.54

Means:
36.1 U/mL (IMMULITE 2000)
36.9 U/mL (IMMULITE)

II. Analytical Performance

See Tables and Graphs for data *representative* of the assay's performance. Results are expressed in U/mL (P.E.I.). (Unless otherwise noted, all were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

Calibration Range: 2 – 100 U/mL (P.E.I., Paul Ehrlich Institute).

Precision: Serum samples were assayed in duplicate over the course of 20 days, two runs per day, for a total of 40 runs and 80 replicates at three US sites. The same design was used for three lots and at three sites. (See "Precision" tables.)

EDTA, heparin, and sodium citrate samples were assayed in duplicate in three runs on three days at three U.S. sites for three lots of IMMULITE Anti-HBc IgM and one of IMMULITE 2000 Anti-HBc

IgM. The median total variance of coefficients (EDTA, 7.7%; heparin, 7.2%; sodium citrate, 8.3%) demonstrated that these alternative sample types do not affect the precision of IMMULITE and IMMULITE 2000 Anti-HBc IgM.

Alternate Sample Types: The measurement of anti-HBc IgM is not significantly affected by the presence of heparinized, sodium citrate, or EDTA anti-coagulants, as shown in a study that included 44 specimens collected into plain, heparinized, sodium citrate, and EDTA vacutainer tubes. By regression:

(Heparin) = 1.02 (Serum) – 0.59 U/mL
r = 0.99

(NaCitrate) = 1.06 (Serum) – 0.71 U/mL
r = 0.94

(EDTA) = 0.99 (Serum) – 0.22 U/mL
r = 0.99

Means:
19.2 U/mL (Serum)
19.1 U/mL (Heparin)
19.5 U/mL (NaCitrate)
18.8 U/mL (EDTA)

(See "Alternate Sample Types Graphs".)

Bilirubin: Presence of unconjugated bilirubin in concentrations up to 20 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 10 and 20 mg/dL of bilirubin concentrations. Performance was not established with clinical specimens.

Hemolysis: Presence of hemoglobin in concentrations up to 540 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 168, 252 and 504 mg/dL of hemoglobin concentrations. Performance was not established with clinical specimens.

Lipemia: Presence of lipemia in concentrations up to 3,000 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 500, 1,000, 2,000 and 3,000 mg/dL of lipemia triglycerides. Performance was not established with clinical specimens.

Analytical Specificity: Analytical specificity was evaluated at two clinical sites in the United States and at one European site. In the United States, serum specimens from 17 subgroups of patients with potentially cross-reacting microorganisms or conditions were tested by IMMULITE Anti-HBc IgM and a commercially available microparticle enzyme immunoassay for anti-HBc IgM (Kit A), with the following results:

	Kit A				
	+		–[1]		
	IMMULITE Anti-HBc IgM				
Sample type	+	–	+	–	Total
HAV	0	0	1	46	47
HCV	0	0	4	68	72
HDV	0	0	0	21	21
HEV	0	0	0	6	6
Non-viral liver diseases[2]	0	0	1	53	54
Autoimmune diseases	0	0	0	25	25
CMV	0	0	0	13	13
EBV	0	0	0	19	19
Syphilis	0	0	0	11	11
Toxoplasma	0	0	0	20	20
HSV	0	0	0	47	47
Parvovirus B19	0	0	0	12	12
HIV	0	1	1	49	51
Influenza vaccine recipient	0	0	0	25	25
Transplant recipient	0	0	0	14	14
Dialysis	0	0	0	33	33
Intravenous drug abuser	0	0	0	5	5
Others[3]	2	0	1	3	6
Total	2	1	8	470	481

[1] Includes Indeterminate cases.

[2] Carcinoma, cirrhosis, hepatorenal disease, fatty liver, passive congestion, cholangitis, biliary obstruction, drug induced hepatitis, choledocholithiasis.

[3] Patients with preclampsia, vasculitis, and sarcoidosis.

In the European study, four rubella IgM positive specimens, six antinuclear antibody (ANA) positive specimens, and 26 rheumatoid factor (RF) positive specimens were tested by IMMULITE Anti-HBc IgM. IMMULITE Anti-HBc IgM test results were negative for all specimens.

32

References

1 Locarnini SA, Gust ID. Hepadnaviridae: hepatitis B virus and the delta virus. In: Balows A, et al, editors. Laboratory diagnosis of infectious diseases: principles and practices. New York: Springer-Verlag, 1988: 750-96.

2 Follett EAC. Diagnosis of hepatitis B infection. In: Young H, McMillan A, editors. Immunological diagnosis of sexually transmitted diseases. New York: Marcel Dekker, 1988: 433-49.

3 Hollinger FB, Dienstag JL. Hepatitis B and D viruses. In: Lennette EH, et al, editors. Manual of clinical microbiology. 6th ed. Washington, D.C.: American Society for Microbiology, 1995:1033-49.

4 Nowicki MJ, Balistreri WF. Hepatitis A to E: building up the alphabet. Contemporary Peds 1992: 118-28.

5 Zuckerman AJ, et al. Hepatitis B virus and hepatitis D virus. In: Principles and practice of clinical virology. 2nd ed. New York: John Wiley & Sons, 1992: 153-72.

6 Tietz NW, editor. Clinical guide to laboratory tests. 3rd ed. Philadelphia: WB Saunders, 1995:354-60.

7 Tietz NW, editor. Clinical guide to laboratory tests. 3rd ed. Philadelphia: W.B. Saunders, 1995:322-4.

8 National Committee for Clinical Laboratory Standards. Procedures for the collection of diagnostic blood specimens by venipuncture; approved standard. 4th ed. NCCLS Document H3-A4, Wayne, PA: NCCLS, 1998.

9 NCCLS. *Internal Quality Control: Principles and Definitions; Approved Guideline.* NCCLS document C24-A (ISBN 1-56238-112-1). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087; 1991.

10 NCCLS: *Procedures for the Handling and Processing of Blood Specimens; Approved Guideline-Second Edition.* NCCLS document H18-A2 (ISBN 1-56238-388-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087; 1999.

11 Code of Federal Regulations, Title 42, Part 493.1201-493.1285, Subpart K-*Quality Control for Tests of Moderate Complexity,* Volume 3. U.S. Government Printing Office; 1993.

Technical Assistance

In the United States, contact DPC's Technical Services department. Tel: 800.372.1782 or 973.927.2828 Fax: 973.927.4101. Outside the United States, contact your National Distributor.

The Quality System of Diagnostic Products Corporation is registered to ISO 9001:1994.

Tables and Graphs

Precision (U/mL)

Site 1

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	6.44	0.27	4.2%	0.36	5.6%
2	10.5	0.34	3.3%	0.47	4.5%
3	56	3.68	6.6%	3.58	6.4%
4	58	2.06	3.6%	2.75	4.8%
5	76	2.48	3.3%	3.01	4.0%

Site 2

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	6.37	0.21	3.2%	0.36	5.6%
2	9.52	0.24	2.5%	0.53	5.6%
3	50.1	3.78	7.5%	3.87	7.7%
4	57	1.38	2.4%	3.25	5.7%
5	74	2.88	3.9%	5.18	7.0%

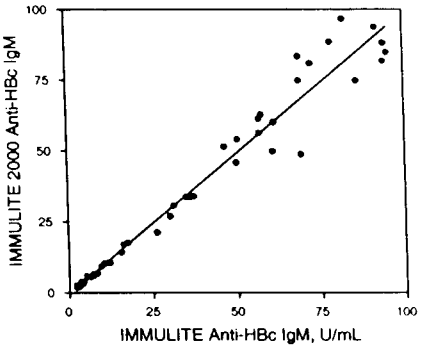
Site 3

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	6.41	0.20	3.2%	0.37	5.8%
2	10.29	0.30	2.9%	0.60	5.8%
3	48.8	2.89	5.9%	3.70	7.6%
4	58	2.02	3.5%	2.51	4.3%
5	76	2.62	3.5%	2.99	4.0%

Lot-to-Lot and Site-to-Site Precision

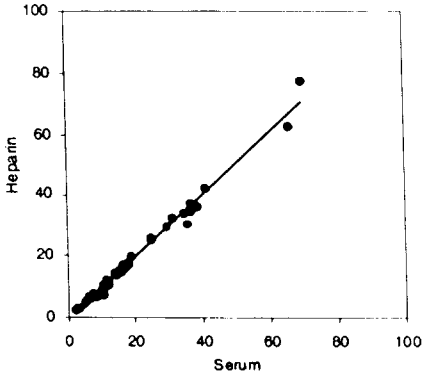
		Lot-to-Lot		Site-to-Site	
	Mean	SD	CV	SD	CV
1	6.40	0.47	7.4%	0.47	7.4%
2	9.98	0.73	7.3%	0.79	7.9%
3	50.6	4.53	9.0%	5.91	11.7%
4	56.0	3.60	6.4%	3.61	6.4%
5	74.0	4.50	6.1%	4.55	6.2%

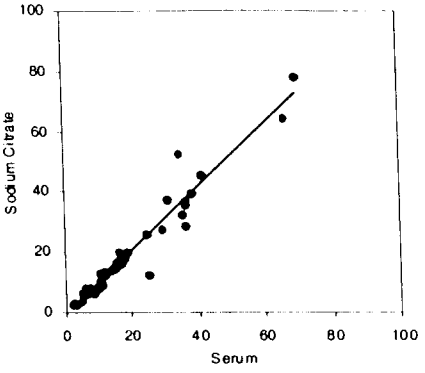
Method Comparison (U/mL)

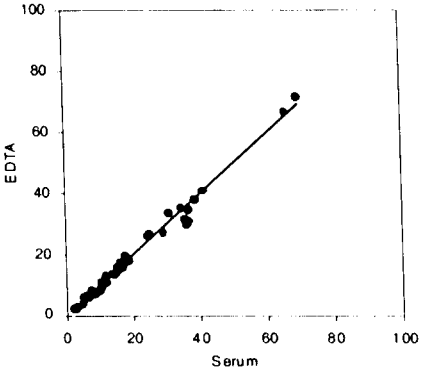


(IML 2000) = 0.99 (IML) – 0.34 U/mL
r = 0.981

Alternate Sample Types Graphs (U/mL)







DPC®
Diagnostic Products Corporation
5700 West 96th Street
Los Angeles, CA 90045-5597

2002-07-30 (ISO 8601)

December 13, 2002

PILKMC – 7

IMMULITE 2000

Anti-HBc IgM

IgM Antibodies to Hepatitis B Core Antigen

DPC

35

IMMULITE® 2000 Anti-HBc IgM

WARNING

This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

Performance characteristics have not been adequately established for use of the IMMULITE 2000 Anti-HBc IgM assay in infants, children, or adolescents.

Federal law restricts this device to sale by or on the order of a physician.

Assay performance characteristics have not been established when the IMMULITE 2000 Anti-HBc IgM assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

Intended Use: IMMULITE 2000 Anti-HBc IgM is a solid-phase chemiluminescent enzyme immunoassay designed for use on the IMMULITE 2000 automated immunoassay analyzer for the qualitative measurement of IgM antibody to hepatitis B core antigen (anti-HBc IgM) in human serum or plasma (EDTA, heparinized, citrate). It is intended for *in vitro* diagnostic use for the presumptive laboratory diagnosis of acute or recent (usually within six months) hepatitis B viral infection.

Caution: Performance characteristics for the IMMULITE 2000 Anti-HBc IgM were determined in part using archival specimens. Laboratories are advised that they should monitor results using other DPC HBV serological markers or retest questionable specimens with IMMULITE 2000 Anti-HBc IgM or another legally-marketed Anti-HBc IgM assay.

Catalog Number: **L2KMC2** (200 tests)

Test Code: **BcM** Color: **Aqua**

Summary and Explanation

Hepatitis B virus (HBV) is the sole human pathogen in the family Hepadnaviridiae, a DNA virus, and is found worldwide. Distribution of HBV infection will vary among geographical areas and population groups. Transmission of the virus is due to parenteral contact, through the exchange of blood or blood products, sexual contact, and perinatal spread from mother to newborn.[1,2] Clinical manifestations range from mild asymptomatic infections to severe fulminant hepatitis.[1,2,3 3] Over 90% of infected adults will have an acute self-limiting infection,[2] with approximately 30% of these individuals developing jaundice, liver enzyme elevation, and symptoms. Recovery occurs without any chronic sequelae.[1,2]

Chronic liver disease, a condition in which infection persists for more than six months, a known sequela of a hepatitis B infection, is usually progressive.[1,2] The risk of developing the chronic carrier state is more likely to follow infection acquired in childhood than as an adult.[4,5] In chronic HBV carriers, there is no evidence of continued hepatic damage,[1,2] however, the infection persists and the carrier maintains the ability to transmit the virus.[2]

Availability of HBV vaccines, and the recommendation of universal immunization for infants and other high-risk persons has aided in the prevention of HBV infections. In addition, treatment with alpha-interferon to relieve symptoms is available. Results have shown positive response to treatment in 40–50% of selected individuals with chronic active hepatitis B.[4,5]

Classification of a hepatitis B infection requires the identification of several serological markers expressed during three phases (incubation, acute and convalescent) of the infection. The first marker to appear during the incubation phase is HBsAg, and indicates an ongoing infection with HBV.[1,2,4] IgM and total anti-HBc appears shortly after the appearance of HBsAg, when the individual usually becomes symptomatic, and peaks during the acute phase prior to the appearance of anti-HBs. IgM antibody to the core antigen will decline in uncomplicated acute infection, whereas IgG antibody will persist for years.[4,5] IgM and total anti-HBc may also be elevated in chronic HBV infections.[4]

Presence of IgM and total anti-HBc indicates an ongoing or recent HBV infection. When used in conjunction with tests for other HBV serological markers, a

36

laboratory diagnosis or a rule out of HBV infection can be achieved.

Principle of the Procedure

IMMULITE 2000 Anti-HBc IgM is a solid-phase, two-step chemiluminescent enzyme IgM antibody capture immunoassay. The solid phase, a polystyrene bead enclosed within a Test Unit, is coated with a monoclonal murine anti-IgM antibody.

The patient specimen is added to the Test Unit containing a coated bead. An alkaline phosphatase-labeled recombinant HBc antigen is also added to the Test Unit. After the wash and incubation steps, chemiluminescent substrate undergoes hydrolysis in the presence of alkaline phosphatase. IMMULITE 2000 Anti-HBc IgM is an immunometric assay. The photon output, as measured by the luminometer, is related to the presence of HBc IgM antibodies in the sample.

Incubation Cycles: 2 × 30 minutes.

Specimen Collection

Hemolyzed samples may indicate mistreatment of a specimen before receipt by the laboratory; hence, the results should be interpreted with caution.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Samples should be thoroughly separated from all cellular material. Failure to do so may lead to a falsely elevated result.[9]

The IMMULITE 2000 Anti-HBc IgM assay may be performed on human serum or plasma (heparinized, sodium citrate, or EDTA).

If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.

Specimens containing precipitate may give inconsistent test results. To prevent this problem, such specimens should be clarified prior to assaying. Specimens showing particulate matter or red blood cells may give inconsistent results and should be centrifuged prior to testing (recommended 8,000 to 10,000 RCF × 10 minutes). Specimens, which are not tested within 24 hours, should be removed from the clot or red blood cells.

Do not use heat-inactivated specimens.

Specimens with obvious microbial contamination should not be tested.

More than two freeze-thaw cycles are not recommended.

Volume Required: 10 µL serum or plasma (heparinized, sodium citrate or EDTA).

Storage: 2 days at room temperature (15°–28°C).[10]
3 days at 2–8°C.[6,10]
For longer storage: at –20°C.[7]

Automatic Predilution Factor: 20. Dispense required volume of diluent (L2MCZ1) to a suitable test tube with barcode label applied.

Warnings and Precautions

For *in vitro* diagnostic use.

This assay was designed and validated for use with human serum or EDTA, heparin, and Na Citrate plasma from individual patient specimens. Pooled specimens must not be used.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Because no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents, specimens should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories, 1993.*

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested by FDA recommended test procedures and found nonreactive for

37

syphilis; for antibodies to HIV1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

The Anti-HBc IgM Adjustor, Anti-HBc IgM Low Positive and Anti-HBc IgM Positive Controls contain HBcAg which may be indicative of low levels of HBV. These components have been inactivated by proven, documented methods. However, always handle all controls as if capable of transmitting infectious agents.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water for the wash step and to avoid system contamination.

Materials Supplied

The principle components — Bead Pack, Reagent Wedge, and Adjustors — represent a matched set. Barcodes encode information about the test, including expiration dates, component lot numbers, adjustment parameters, and cutoff parameters.

Anti-HBc IgM Bead Pack (L2MC12)
With barcode. 200 beads coated with a monoclonal murine anti-IgM antibody manufactured at DPC. Stable at 2–8°C until expiration date.

Anti-HBc IgM Reagent Wedge (L2MCA2)
With barcode. 11.5 mL of a protein-based buffer, with preservative. 11.5 mL alkaline phosphatase (bovine calf intestine) conjugated to a purified recombinant HBcAg (produced in *E. coli* bacteria) in buffer, with < 0.1 g/dL sodium azide. Stable at 2–8°C until expiration date.

Before use, tear off the top of the label at the perforations without damaging the barcode on the main label. Remove the foil seal from the top of the Reagent Wedge, and snap the sliding cover down into the ramps on the reagent lid.

Anti-HBc IgM Adjustors (LMCL, LMCH)
Two vials (Low and High) of lyophilized human serum reactive to HBcAg in buffer, with < 0.1 g/dL sodium azide. Reconstitute each vial with **2.0 mL** distilled or deionized water. Mix by gentle swirling or inversion until the lyophilized material is fully dissolved. (No further dilution is required.) Stable at 2–8°C for 14 days after reconstitution, or for 6 months (aliquoted) at –20°C.

Anti-HBc IgM Controls (LMCC1, LMCC2, LMCC3) (may be purchased separately.)
Three vials (Negative, Low Positive and Positive). **LMCC1 (Negative Control):** human serum nonreactive to HBcAg, with < 0.1 g/dL sodium azide. **LMCC2, LMCC3 (Low Positive Control, Positive Control):** human serum reactive to HBcAg, with < 0.1 g/dL sodium azide. Reconstitute each vial with **2.0 mL** distilled or deionized water. Mix by gentle swirling or inversion until the lyophilized material is fully dissolved. (No further dilution is required.) Stable at 2–8°C for 14 days after reconstitution, or for 6 months (aliquoted) at –20°C.

For the current control ranges, please refer to the Control insert.

Aliquot Labels with barcodes are supplied with the kit, for use with the Adjustors and Controls. Before use, place the appropriate Aliquot Labels on test tubes, so the barcodes can be read by the barcode reader on the IMMULITE 2000.

Anti-HBc IgM Sample Diluent (L2MCZ1)
For the on-board dilution of patient samples. Concentrated (ready-to-use) buffer solution, with < 0.1 g/dL sodium azide. Stable at 2–8°C for 30 days after opening, or 6 months (aliquotted) at –20°C.
L2MCZ1: 25 mL.

Barcode labels are provided for use with the diluent. Before use, place an appropriate label on a 16 x 100 mm test tube, so that the barcode can be read by the on-board reader.
L2MCZ1:3 labels

Kit Components Supplied Separately

L2SUBM: Chemiluminescent Substrate
L2PWSM: Probe Wash
L2KPM: Probe Cleaning Kit
L2RXT: Reaction Tubes (disposable)
L2ZT: 250 Sample Diluent Test Tubes (16 x 100 mm)
L2ZC: 250 Sample Diluent Tube Caps

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in Section 3 of the IMMULITE 2000 Operator's Manual.

See the IMMULITE 2000 Operator's Manual Section 6 for routine operation procedures (preparation, setup, assay, and quality control) and Section 4 for the adjustment procedure.

Adjustment Interval: 4 weeks. . The adjustors are used to correlate the cps (counts per minute) of the customer's instrument to that of the master curve instrument.

Quality Control Samples: The control(s) supplied with the kit should be used as quality control material to monitor the performance of the assay.

The quality control material is in a serum matrix. It may not adequately control the assay for plasma matrix specimens. The user should provide alternate control material for a plasma matrix.

Note that when analyzing samples under the "Control" of IMMULITE 2000 software, no auto-dilution is performed. Anti-HBc IgM Controls (LMCC1-3) are supplied pre-diluted and require no auto-dilution.

If control results fall outside the stated range, patient results should not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.

It is recommended that the user refer to *Internal Quality Control Testing: Principles and Definitions* and other published guidelines for general quality control recommendations and intervals for testing.[9,11]

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

The low positive control is used to verify that the test kit components are capable of detecting a reactive specimen, provided the test procedure has been strictly adhered to.

It is recommended that the controls supplied with the kit be used as quality control material to monitor the performance of the assay.

For the current control ranges, please refer to the Control insert.

Calibration

The IMMULITE 2000 Anti-HBc IgM calibration employs a stored master curve, generated by the four-parameter logistic mathematical model based on the dose-cps (counts per minute) relationship during the calibration process.

DPC's IMMULITE 2000 Anti-HBc IgM is calibrated against the Anti-HBc IgM reference serum from Paul Ehrlich Institute (PEI). The cutoff of the assay was set at 10 U/mL based on an ROC analysis from 130 anti-HBc IgM positive (spiked and endogenous) and negative (endogenous) specimens and on subsequent cutoff validation studies.

Interpretation of Results

Positive: A result greater than, or equal to 11 U/mL is considered to be "presumptively positive" for IgM antibody to HBcAg, and is presumptive evidence of recent infection with HBV. Further testing is required with other HBV serological markers to confirm the HBV infection state.

Negative: A result less than 9 U/mL is considered to be "negative" and is indicative that the patient has not been recently infected with HBV. Negative results by this test do not preclude recent primary infection. To define the HBV infection state further testing is recommended with other HBV serological markers.

Retest Zone: A result less than 11 U/mL and greater than or equal to 9 U/mL is indeterminate and must be retested in duplicate. If all three results (original and two retests) are greater than or equal to 10 U/mL, the specimen is positive for anti-HBc IgM. If all three results (original and two retests) are less than 10 U/mL, the specimen is negative for anti-HBc IgM.

A specimen that has been retested but cannot be resolved as above remains indeterminate. An indeterminate result (9-11 U/mL) should not be reported until it is retested and resolved. The immune status of the individual should be further assessed by associated risk factors and the use of additional diagnostic information, or another sample may be collected and tested.

It is recommended that patients exhibiting indeterminate IMMULITE 2000 Anti-HBc IgM results be closely monitored over time (approximately one week intervals) to determine if the IgM anti-HBc result becomes reactive, i.e., >11 (associated with acute hepatitis B infection), or non-reactive, i.e., <9 (associated with recovery).

For the determination of seroconversion, two sera or plasma (heparinized, sodium citrate or EDTA) samples should be drawn three to four weeks apart, during the acute and convalescent stages of the infection.

If U/mL values are reported, it is recommended that the following be included with the report:

> The following results were obtained with the IMMULITE 2000 IgM Anti-HBc EIA. Values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported total IgM anti-HBc level cannot be correlated to an endpoint titer. The clinical significance of values reported greater than or equal to 11 mIU/mL and less than 9 mIU/mL have not been determined, other than the individual is presumed to have been recently infected with HBV (≥ 11) or has not been recently infected with HBV (<9).

Limitations

The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall laboratory and clinical picture. False results may be obtained with any diagnostic test.

IMMULITE 2000 Anti-HBc IgM is limited to the detection of IgM anti-HBc in human serum or plasma. It can be used to furnish presumptive information as to whether a patient has, or has recently had, acute or subclinical hepatitis B infection. Supportive laboratory and clinical information, including other hepatitis B markers, must be used to determine the HBV disease state.

Assay performance characteristics have not been established for any specimen matrices other than serum, or heparinized, EDTA, or sodium citrate plasma.

Testing using alternative methodologies may be warranted if signs, symptoms, and risk factors are indicative of viral hepatitis and other laboratory tests are nonreactive for the diagnosis of viral hepatitis.

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of this type of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

Expected Values

Individuals acutely infected with the hepatitis B virus exhibit anti-HBc IgM between two weeks and four months after infection, usually through the course of clinical illness. IgM antibody levels decline in cases of uncomplicated acute infection, but remain elevated in chronic HBV infection.

Demographics and expected prevalence rates for apparently healthy, pre-vaccination, and pregnant subjects, each of whom provided one specimen, from three clinical studies, one in the northwestern United States (Study 1), one in the southern United States (Study 4)

and one in Europe, are summarized in the following table.

Subject	Total *n*	Male	Female	Mean Age	Age Range	Pos. by IML 2000 Anti-HBc IgM	
						n	%
Apparently Healthy (Europe)	105	Not available				0	0.0%
Pre-vaccination (Study 1)	17	8	9	34	21-56	0	0.0%
Pregnant at high risk[1] (Study 1)	3	0	3	33	30-36	1	33.3%
Pregnant at low risk[1] (Study 1)	16	0	16	31	21-42	0	0.0%
Pregnant at low risk[1] (Study 4)	200	0	200	28	17-41	1	0.5%
Pregnant at low risk[1] (Europe)	13	Not available				0	0.0%
HB acute patients (Study 1)	2	1	1	46	39-53	2	100%
HB acute patients (Europe)	114	Not available				109	95.6%

[1] At high risk or low risk of exposure to hepatitis B.

Performance Characteristics

I. Clinical Performance

Four clinical studies with 769 specimens and one comparison study with 46 specimens from a total of 815 subjects were conducted to assess the performance of the IMMULITE 2000 Anti-HBc IgM assay.

The participating subjects each contributed one specimen to the analyses. The specimens were tested by FDA-approved or licensed hepatitis B assays (Reference markers) and IMMULITE 2000 Anti-HBc IgM. All specimens in the analyses were initial test results.

The data were analyzed following the assignment of specimen classification based upon the reactive(+)/ nonreactive(-) patterns for the HBV reference serological markers. Specimen classification was based only on the HBV serological marker results for that particular specimen. No other laboratory or clinical information was used in the disease classification process.

Study 1: Conducted in the northwestern United States, this study included 281 patients, consisting of 138 males and 88 females. Gender for 55 subjects was not reported. These subjects had an average age of 43 years, ranging from 21 to 85 years. Distributions of the ethnicity of the subjects are shown in the following table.

Ethnicity	*n*	%
African American	15	5.3%
Caucasian	169	60.1%
Hispanic	2	0.7%
Asian	32	11.4%
Other	3	1.1%
Unknown	60	21.4%
Total	281	100.0%

These subjects included acute and chronic hepatitis B patients, vaccinated individuals, pregnant women, and apparently healthy individuals, and patients with potentially crossreactive substances and medical conditions.

A total of 281 specimens were prospectively (n=92) and retrospectively (n=189) collected and tested by FDA-approved or licensed hepatitis B assays for six HBV serological markers (HBsAg, HBeAg, anti-HBc IgM, anti-HBc, anti-HBe, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated 17 unique patterns:

41

Characterization based on single point specimens	No. of patients	HBV Reference Markers					
		HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc	Anti-HBe	Anti-HBs
Acute	2	+	–	–	–	–	–
Acute	1	+	+	–	–	–	–
Acute	32	+	–	+/-	+	+	–
Acute	34	+	+	+/-	+	–	–
Chronic	2	+	–	–	+	–	–
Chronic	3	+	+/-	–	+	+	+
Chronic	1	+	–	–	+	–	+
Chronic	1	+	+	+/-	+	–	+
Early Recovery	16	–	–	+/-	+	+	+
Early Recovery	4	–	–	–	+	+	–
Early Recovery	19	–	–	–	+	+/-	–
Early Recovery	1	–	–	+	+	+/-	+
HBV vaccine response	27	–	–	–	–	–	+
Not previously infected	120	–	–	–	–	–	–
Recovered	16	–	–	–	+/-	–	+
Recovered	1	–	+/-	–	+	–	+
Uninterpretable	1	–	+	–	–	–	–

Based on the above classifications the IMMULITE 2000 Anti-HBc IgM results were compared to Kit A, a reference assay for the determination of anti-HBc IgM.

Reference	Kit A						Total
	+		Ind		–		
	IML 2000 Anti-HBc IgM						
	+	–	+	–	+	–	
HB Acute infection	6	0	3	0	9	51	69
HB Chronic infection	0	0	0	0	1	6	7
HB Early recovery	0	1	1	0	0	38	40
HBV Vaccine response	0	0	0	0	0	27	27
Not previously infected	0	0	0	0	1	119	120
Recovered	0	0	0	0	2	15	17
Uninterpretable	0	0	0	0	0	1	1
Total	6	1	4	0	13	257	281

HB Acute Infection
Positive agreement = 100.0% (6/6)
95% CI = 54.1% to 100.0%
Negative agreement = 85.0% (51/60)
95% CI = 73.4 to 92.9%

HB Chronic Infection
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 85.7% (6/7)
95% CI = 42.1 to 99.6%

Early Recovery
Positive agreement = N/A (0/1)
95% CI = N/A
Negative agreement = 100.0% (38/38)
95% CI = 90.7 to 100.0%

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (27/27)
95% CI = 87.2 to 100.0%

Not previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 99.2% (119/120)
95% CI = 95.4 to 100.0%

Recovered
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 88.2% (15/17)
95% CI = 63.6 to 98.5%

Uninterpretable
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (1/1)
95% CI = N/A

Total
Positive agreement = 85.7% (6/7)
95% CI = 42.1 to 99.6%
Negative agreement = 95.2% (257/270)
95% CI = 91.9 to 97.4%
Total agreement = 94.9% (263/277)
95% CI = 91.7 to 97.2%

Study 2: Conducted in the northeastern United States, this study included 209 patients, consisting of 104 males and 103 females. Gender for two patients was not reported. These patients had an average age of 47 years, ranging from newborn to 93 years. Distributions of the ethnicity of the patients are shown in the following table.

Ethnicity	*n*	%
African American	21	10.0%
Caucasian	101	48.3%
Hispanic	3	1.4%
Asian	6	2.9%
Other	7	3.3%
Unknown	71	34.0%
Total	209	100.0%

Included were patients with potentially crossreactive substances and medical conditions.

A total of 209 retrospective specimens were collected and tested by FDA-approved or licensed hepatitis B assays for four HBV serological markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated eight unique patterns:

Characterization based on single point specimens	# of patients	HBV Reference Markers			
		HBsAg	Anti-HBc IgM	Anti-HBc	Anti-HBs
Acute	8	+	–	–	–
Acute	9	+	+/-	+	–
Chronic	2	+	–	+	+
Early recovery	33	–	+/-	+	+
Early recovery	17	–	–	+	–
HBV vaccine response	32	–	–	–	+
Not previously infected	107	–	–	–	–
Uninterpretable	1	+	–	–	+

Based on the above classifications the IMMULITE 2000 Anti-HBc IgM results were compared to Kit A.

Reference Serological Characterization	Kit A						Total
	+	+	Ind	Ind	–	–	
	IML 2000 Anti-HBc IgM						
	+	–	+	–	+	–	
HB Acute infection	2	0	1	0	1	13	17
HB Chronic infection	0	0	0	0	0	2	2
HB Early recovery	0	0	2	0	2	46	50
HBV Vaccine response	0	0	0	0	0	32	32
Not previously infected	0	0	0	0	0	107	107
Uninterpretable	0	0	0	0	0	1	1
Total	2	0	3	0	3	201	209

HB Acute Infection
Positive agreement = N/A (2/2)
95% CI = N/A
Negative agreement = 92.9% (13/14)
95% CI = 66.1 to 99.8%

HB Chronic Infection
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (2/2)
95% CI = N/A

Early Recovery
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 95.8% (46/48)
95% CI = 85.7 to 99.5%

43.

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (32/32)
95% CI = 89.1 to 100.0%

Not previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (107/107)
95% CI = 96.6 to 100.0%

Uninterpretable
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (1/1)
95% CI = N/A

Total
Positive agreement = N/A (2/2)
95% CI = N/A
Negative agreement = 98.5% (201/204)
95% CI = 95.8 to 99.7%
Total agreement = 98.5% (203/206)
95% CI = 95.8 to 99.7%

Study 3: Specimens obtained from China were tested in the southern United States. This study included 79 patients and was comprised of 13 females and 55 males (gender for 11 patients was not reported) with an average age of 36 years, ranging from 18 to 82 years. These were prospectively recruited patients from a clinically well-characterized, homogeneous population: acute hepatitis B patients who presented with symptoms typical of acute hepatitis B such as jaundice, persistent fatigue, loss of appetite, pale stools and liver enlargement. Their ALT and AST results were significantly elevated at the time of diagnosis.

A total of 79 specimens were prospectively collected and tested by FDA-approved or licensed hepatitis B assays for six HBV serological markers (HBsAg, HBeAg, anti-HBc IgM, anti-HBc, anti-HBe, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated 11 unique patterns:

Characterization based on single point specimens	# pat	HBV Reference Markers					
		HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc	Anti-HBe	Anti-HBs
Acute	23	+	–	+/-	+	+	–
Acute	8	+	+/-	+	+	+	–
Acute	1	+	–	+	+/-	–	–
Acute	35	+	+	+/-	+	–	–
Acute	4	+	+	+	+	–	+/-
Chronic	1	+	–	–	+	–	–
Chronic	3	+	+/-	–	+	+	–
Chronic	1	+	+	+/-	+	–	+
Chronic	1	+	+	+	+	+	+
Recovered	1	–	–	–	+/-	–	+
Uninterpretable	1	+	–	+	+	+	+

Based on the above classifications the IMMULITE 2000 Anti-HBc IgM results were compared to Kit B, a reference assay for the determination of anti-HBc IgM.

Reference Serological Characterization	Kit B						Total
	+		Ind		–		
	IML 2000 Anti-HBc IgM						
	+	–	+	–	+	–	
HB Acute infection	30	3	3	0	13	22	71
HB Chronic infection	1	0	0	0	3	2	6
Recovered	0	0	0	0	0	1	1
Uninterpretable	1	0	0	0	0	0	1
Total	32	3	3	0	16	25	79

HB Acute Infection
Positive agreement = 90.9% (30/33)
95% CI = 75.7 to 98.1%
Negative agreement = 62.9% (22/35)
95% CI = 44.9 to 78.5%

HB Chronic Infection
Positive agreement = N/A (1/1)
95% CI = N/A
Negative agreement = 40.0% (2/5)
95% CI = 5.3 to 85.3%

Recovered
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (1/1)
95% CI = N/A

Uninterpretable
Positive agreement = N/A (1/1)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

Total
Positive agreement = 91.4% (32/35)
95% CI = 76.9 to 98.2%
Negative agreement = 61.0% (25/41)
95% CI = 44.5 to 75.8%
Total agreement = 75.0% (57/76)
95% CI = 63.7 to 84.2%

Study 4: Conducted in the southern United States, this study included retrospectively collected specimens from 200 pregnant subjects. These subjects had an average age of 28 years, ranging from 17 to 41 years.

A total of 200 specimens were tested by FDA-approved or licensed hepatitis B assays for four HBV serological markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated three unique patterns:

Characterization based on single point specimens	No. of subjects	HBV Reference Markers			
		HBsAg	Anti-HBc IgM	Anti-HBc	Anti-HBs
Early recovery	4	–	+/-	+	+
Early recovery	2	–	–	+	–
HBV vaccine response	42	–	–	–	+
Not previously infected	152	–	–	–	–

Based on the above classifications the IMMULITE 2000 Anti-HBc IgM results were compared to Kit B.

Reference Serological Characterization	Kit B						Total
	+		Ind		–		
	IML 2000 Anti-HBc IgM						
	+	–	+	–	+	–	
Early recovery	0	0	0	0	1	5	6
HBV vaccine response	0	0	0	0	0	42	42
Not previously infected	0	0	0	0	0	152	152
Total	0	0	0	0	1	199	200

Early Recovery
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 83.3% (5/6)
95% CI = 35.9 to 99.6%

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (42/42)
95% CI = 91.6 to 100.0%

Not Previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (152/152)
95% CI = 97.6 to 100.0%

Total
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 99.5% (199/200)
95% CI = 97.2 to 100.0%
Total agreement = 99.5% (199/200)
95% CI = 97.2 to 100.0%

Study 5: In an additional study conducted in-house at DPC, IMMULITE 2000 Anti-HBc IgM was compared to DPC's IMMULITE Anti-HBc IgM on 46 samples. (Concentration range: approximately 2 to 95 U/mL. See "Method Comparison" graph.) By linear regression:

(IML 2000) = 0.99 (IML) – 0.34 U/mL
r = 0.981

Slope 95% CI: 0.93, 1.05

Intercept 95% CI: -3.22, 2.54

Means:
36.1 U/mL (IMMULITE 2000)
36.9 U/mL (IMMULITE)

II. Analytical Performance

See Tables and Graphs for data *representative* of the assay's performance. Results are expressed in U/mL (P.E.I.). (Unless otherwise noted, all were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

Calibration Range: 2 – 100 U/mL (P.E.I., Paul Ehrlich Institute).

Precision: Serum samples were assayed in duplicate over the course of 20 days, two runs per day, for a total of 40 runs and 80 replicates at three US sites.

(See "Precision" tables.)

Caution: ***The IMMULITE 2000 Anti-HBc IgM lot-to-lot precision has not been evaluated.***

45

EDTA, heparin, and sodium citrate samples were assayed in duplicate in three runs on three days at three U.S. sites for three lots of IMMULITE Anti-HBc IgM and one lot of IMMULITE 2000 Anti-HBc IgM. The median total variance of coefficients (EDTA, 7.7%; heparin, 7.2%; sodium citrate, 8.3%) demonstrated that these alternative sample types do not affect the precision of IMMULITE and IMMULITE 2000 Anti-HBc IgM.

Alternate Sample Types: The measurement of anti-HBc IgM is not significantly affected by the presence of heparinized, sodium citrate, or EDTA anti-coagulants, as shown in a study that included 44 specimens collected into plain, heparinized, sodium citrate, and EDTA vacutainer tubes. By regression:

(Heparin) = 1.03 (Serum) – 0.56 U/mL
r = 1.00

(NaCitrate) = 1.05 (Serum) – 0.77 U/mL
r = 0.98

(EDTA) = 1.02 (Serum) – 0.38 U/mL
r = 0.99

Means:
19.5 U/mL (Serum)
19.6 U/mL (Heparin)
19.6 U/mL (NaCitrate)
19.4 U/mL (EDTA)

(See "Alternate Sample Types Graphs".)

Bilirubin: Presence of unconjugated bilirubin in concentrations up to 20 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 10 and 20 mg/dL of bilirubin concentrations. Performance was not established with clinical specimens.

Hemolysis: Presence of hemoglobin in concentrations up to 540 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 168, 252 and 504 mg/dL of hemoglobin concentrations. Performance was not established with clinical specimens.

Lipemia: Presence of lipemia in concentrations up to 3,000 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 500, 1,000, 2,000 and 3,000 mg/dL of lipemia triglycerides. Performance was not established with clinical specimens.

Analytical Specificity: Analytical specificity was evaluated at two clinical sites in the United States and at one European site. In the United States, serum specimens from 17 subgroups of patients with potentially cross-reacting microorganisms or conditions were tested by IMMULITE 2000 Anti-HBc IgM and a commercially available microparticle enzyme immunoassay for anti-HBc IgM (Kit A), with the following results:

	Kit A				
	+		–[1]		
	IML 2000 Anti-HBc IgM				
Sample type	+	–	+	–	Total
HAV	0	0	1	46	47
HCV	0	0	5	67	72
HDV	0	0	1	20	21
HEV	0	0	0	6	6
Non-viral liver diseases[2]	0	0	1	53	54
Autoimmune diseases	0	0	0	25	25
CMV	0	0	0	13	13
EBV	0	0	0	19	19
Syphilis	0	0	0	11	11
Toxoplasma	0	0	0	20	20
HSV	0	0	0	47	47
Parvovirus B19	0	0	0	12	12
HIV	0	1	1	49	51
Influenza vaccine recipient	0	0	0	25	25
Transplant recipient	0	0	0	14	14
Dialysis	0	0	0	33	33
Intravenous drug abuser	0	0	0	5	5
Others[3]	0	2	3	1	6
Total	2	1	10	468	481

[1] Includes Indeterminate cases.

[2] Carcinoma, cirrhosis, hepatorenal disease, fatty liver, passive congestion, cholangitis, biliary obstruction, drug induced hepatitis, choledocholithiasis.

[3] Patients with preclampsia, vasculitis, and sarcoidosis.

46

In the European study, four rubella IgM positive specimens, five antinuclear antibody (ANA) positive specimens, and 23 rheumatoid factor (RF) positive specimens were tested by IMMULITE 2000 Anti-HBc IgM. IMMULITE 2000 Anti-HBc IgM test results were negative for all specimens.

References

1 Locarnini SA, Gust ID. Hepadnaviridae: hepatitis B virus and the delta virus. In: Balows A, et al, editors. Laboratory diagnosis of infectious diseases: principles and practices. New York: Springer-Verlag, 1988: 750-96.

2 Follett EAC. Diagnosis of hepatitis B infection. In: Young H, McMillan A, editors. Immunological diagnosis of sexually transmitted diseases. New York: Marcel Dekker, 1988: 433-49.

3 Hollinger FB, Dienstag JL. Hepatitis B and D viruses. In: Lennette EH, et al, editors. Manual of clinical microbiology. 6th ed. Washington, D.C.: American Society for Microbiology, 1995:1033-49.

4 Nowicki MJ, Balistreri WF. Hepatitis A to E: building up the alphabet. Contemporary Peds 1992: 118-28.

5 Zuckerman AJ, et al. Hepatitis B virus and hepatitis D virus. In: Principles and practice of clinical virology. 2nd ed. New York: John Wiley & Sons, 1992: 153-72.

6 Tietz NW, editor. Clinical guide to laboratory tests. 3rd ed. Philadelphia: WB Saunders, 1995:354-60.

7 Tietz NW, editor. Clinical guide to laboratory tests. 3rd ed. Philadelphia: W.B. Saunders, 1995:322-4.

8 National Committee for Clinical Laboratory Standards. Procedures for the collection of diagnostic blood specimens by venipuncture; approved standard. 4th ed. NCCLS Document H3-A4, Wayne, PA: NCCLS, 1998.

9 NCCLS. *Internal Quality Control: Principles and Definitions; Approved Guideline.* NCCLS document C24-A (ISBN 1-56238-112-1). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087; 1999.

10 NCCLS: *Procedures for the Handling and Processing of Blood Specimens; Approved Guideline-Second Edition.* NCCLS document H18-A2 (ISBN 1-56238-388-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087; 1999.

11 Code of Federal Regulations, Title 42, Part 493.1201-493.1285, Subpart K-*Quality Control for Tests of Moderate Complexity,* Volume 3. U.S. Government Printing Office; 1993.

Technical Assistance

In the United States, contact DPC's Technical Services department.
Tel: 800.372.1782 or 973.927.2828
Fax: 973.927.4101. Outside the United States, contact your National Distributor.

The Quality System of Diagnostic Products Corporation is registered to ISO 9001:1994.

Tables and Graphs

Precision (U/mL)

Site 1

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	6.42	0.21	3.2%	0.29	4.5%
2	10.3	0.35	3.4%	0.51	5.0%
3	56	2.35	4.2%	4.59	8.2%
4	59	1.98	3.4%	4.45	7.6%
5	76	2.75	3.6%	3.57	4.7%

Site 2

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	6.13	0.25	4.0%	0.38	6.1%
2	9.87	0.29	2.9%	0.49	4.9%
3	45.1	1.72	3.8%	2.46	5.5%
4	57	1.25	2.2%	1.80	3.1%
5	74	2.11	2.8%	3.24	4.4%

Site 3

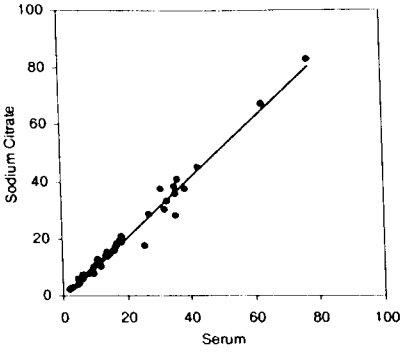
		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	5.57	0.35	6.2%	0.41	7.3%
2	9.65	0.27	2.8%	0.39	4.0%
3	51.2	3.04	5.9%	3.23	6.3%
4	59	1.96	3.3%	2.32	3.9%
5	77	1.90	2.5%	2.54	3.3%

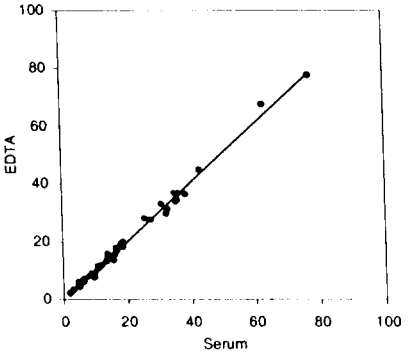
47

Site-to-Site Precision

	Site-to-Site		
	Mean	SD	CV
1	6.04	0.51	8.4%
2	9.93	0.53	5.3%
3	50.7	5.62	11.1%
4	58.3	3.15	5.4%
5	76.0	3.36	4.4%

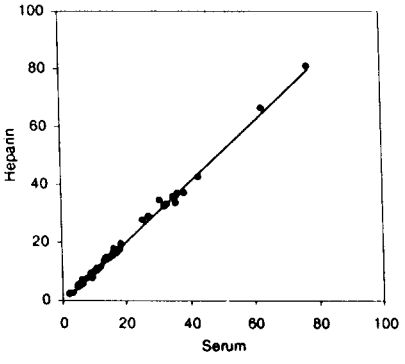
Method Comparison (U/mL)





(IML 2000) = 0.99 (IML) – 0.34 U/mL
r = 0.981

Alternate Sample Types Graphs (U/mL)



Diagnostic Products Corporation
5700 West 96th Street
Los Angeles, CA 90045-5597

2002-07-30 (ISO 8601)

December 13, 2002

PIL2KMC – 11

48